

Table 1. *In vivo* transduction efficiency of nNOS in liver cells from normal and injured livers

Cell Type	Normal	BDL	CCl₄
I1	41.9 ± 3.3	39.2 ± 3.3	37.3 ± 3.1
SEC	63.1 ± 7.8	52.7 ± 4.8 *	41.2 ± 3.6*
HSC	65.9 ± 4.5	50.4 ± 2.9*	40.4 ± 2.7*

Ad.nNOS was administered via the femoral vein (1.5X10¹¹ pfu/kg). For BDL rats, ad.nNOS was infused 2 days after BDL and for CCl₄ injury, virus was given 2 days after the 10th dose. Cell harvest was performed 7 days after adenovirus administration. After cell isolation, cells were allowed to adhere for 18 hours and NADPH-diaphorase staining was performed.

Numbers shown represent percent of total NADPH-diaphorase positive cells (i.e. transduced with nNOS, n=3). *P < 0.05 vs. normal. Abbreviations: I1, hepatocyte; SEC, sinusoidal endothelial cells; HSC, hepatic stellate cell; BDL, bile duct ligation; CCl₄, carbon tetrachloride

Table 2. Relative NO (nitrite) production in normal and injured liver cells after *in vivo* gene transfer

Cell Type	Normal			BDL			CCl ₄		
	Control	Ad-β-gal	Ad.nNOS	Control	Ad-β-gal	Ad.nNOS	Control	Ad-β-gal	Ad.nNOS
H	0.2±0.0	0.2±0.0	1.3±0.3*	0.3±0.0	0.4±0.0	1.3±0.3*	0.2±0.0	0.2±0.0	0.3±0.1*
SEC	5.7±0.1	5.6±0.1	8.9±0.4*	10.8±0.2	11.4±0.3	16.9±0.5*	0.4±0.0	0.4±0.0	2.1±0.4*
HSC	0.4±0.1	0.4±0.1	6.2±0.8*	2.0±0.5	1.9±0.3	4.4±0.8*	0.3±0.1	0.3±0.1	1.3±0.3*

Methods: Liver injury was induced as in Table 1. Ad-β-gal or Ad.nNOS were administered via femoral vein 1.5×10^{11} pfu/kg two days after BDL, or the last dose of CCl₄. Liver cells were isolated 7 days later and were cultured for 24 hours after which nitrite concentrations in conditioned supernatants were determined. Shown are means ± SEM (μM/μg protein). *p<0.05 for Ad.nNOS transfected compared with control or Ad-β-gal transfected (n=3, for each condition). Abbreviations: H, hepatocyte; SEC, sinusoidal endothelial cells; HSC, hepatic stellate cell; BDL, bile duct ligation; CCl₄, carbon tetrachloride

Table 3. Effect of transduced nNOS on portal pressure

Perfusion Pressure (cmH ₂ O)							
Transduction State	Flow Rate (ml/min)				P _{Q=0} (cmH ₂ O)	Slope _{PQR} (cmH ₂ O.min. ml ⁻¹)	
	20	30	40	50			
Normal	4.1 ± 0.2	5.6 ± 0.4	7.4 ± 0.7	10.4 ± 0.5	0.81 ± 0.10	0.15 ± 0.01	
CCl ₄	Ad.β-gal	8.5 ± 0.5 [#]	11.1 ± 0.9 [#]	14.7 ± 0.1 [#]	16.9 ± 0.3 [#]	2.98 ± 0.13 [#]	0.28 ± 0.02 [#]
	Ad.nNOS	6.3 ± 0.2 ^{#*}	8.7 ± 0.3 ^{#*}	12.0 ± 0.2 ^{#*}	14.9 ± 0.4 ^{#*}	1.16 ± 0.03 ^{#*}	0.27 ± 0.01 [#]
BDL	Ad.β-gal	8.8 ± 0.1 [#]	11.0 ± 0.6 [#]	13.6 ± 0.5 [#]	15.4 ± 0.6 [#]	3.32 ± 0.17 [#]	0.25 ± 0.02 [#]
	Ad.nNOS	7.4 ± 0.1 ^{#*}	9.0 ± 0.2 ^{#*}	11.2 ± 0.4 ^{#*}	13.9 ± 0.4 ^{#*}	2.00 ± 0.10 ^{#*}	0.24 ± 0.01 [#]
	7-NI	9.1 ± 0.1 [#]	11.9 ± 0.2 [#]	13.8 ± 0.1 [#]	16.2 ± 0.2 [#]	3.33 ± 0.22 [#]	0.27 ± 0.0 [#]
	7-NI/Ad.nNOS	9.0 ± 0.3 ^{#¶}	11.6 ± 0.2 ^{#¶}	13.5 ± 0.3 ^{#¶}	15.8 ± 0.5 ^{#¶}	3.24 ± 0.10 ^{#¶}	0.26 ± 0.05 [#]

Liver injury (BDL or CCl₄) was as in Example 1. Either Ad.nNOS or Ad.β-gal (each 1.5x10¹¹ pfu/kg) were injected via the femoral vein 7 days prior to isolated liver perfusion. Portal pressure, monitored continuously, was recorded at incremental flow rates and portal resistance was calculated. In experiments where 7-NI was used, this compound was administered by intraperitoneal injection one day prior to BDL (25 mg/kg) and every 2 days thereafter. *p<0.05 vs. normal; #p<0.05 vs. Ad.β-gal for each injury model; ¶p<0.05 vs. Ad.nNOS (all, n=4). Abbreviations: BDL, = bile duct ligation; CCl₄= carbon tetrachloride; P_{Q=0} = flow inlet pressure; Slope_{PQR} = regression slope of multiple point pressure flow relationships; 7-NI = 7-nitroindazole.